

CLAIMS

1. A method of comparing one or more nucleic acid targets within two or more samples, comprising:

- a) preparing a sample mixture by a process comprising obtaining at least a first sample and a second sample, each potentially having at least a first nucleic acid target and mixing the first nucleic acid sample and the second nucleic acid sample to create a sample mixture;
- b) isolating at least a first target fraction of the sample mixture;
- c) performing at least a first amplification reaction on the first target fraction, wherein the amplification reaction produces at least a first amplified nucleic acid, if the first nucleic acid target is present in the first sample, and at least a second amplified nucleic acid, if the first nucleic acid target is present in the second sample;
- d) differentiating the first amplified nucleic acid in the first target fraction, if any, from the second amplified nucleic acid in the first target fraction, if any; and
- e) comparing abundance of the first nucleic acid target of said first sample to abundance of the first nucleic acid target of said second sample.

2. The method of claim 1, wherein the first nucleic acid target is present in the first sample.

3. The method of claim 2, wherein the first nucleic acid target is present in the second sample.

4. The method of claim 1, further defined as comprising performing at least a first amplification reaction on the first target fraction using at least a first target-specific primer, wherein the amplification reaction produces at least a first amplified nucleic acid, if the first

nucleic acid target is present in the first sample, and at least a second amplified nucleic acid, if the first nucleic acid target is present in the second sample.

5. The method of claim 4, further defined as comprising isolating at least a second target fraction from the sample mixture and performing a second amplification reaction on the the second target fraction using a second target-specific primer that is specific for a second target that may be present in the first sample and/or the second sample.

6. The method of claim 1, wherein:

a) preparing the sample mixture is further defined as comprising:

preparing at least a first tagged nucleic acid sample by appending at least a first nucleic acid tag comprising a first amplification domain and a first differentiation domain to the first nucleic acid target of the first sample, if the first nucleic acid target is present in the first sample;

preparing at least a second tagged nucleic acid sample by appending at least a second nucleic acid tag comprising a second amplification domain and a second differentiation domain to the first nucleic acid target of the second sample, if the first nucleic acid target is present in the second sample, wherein the second differentiation domain is different from the first differentiation domain; and

mixing the first tagged nucleic acid sample and the second tagged nucleic acid sample to create the sample mixture; and

b) performing at least a first amplification reaction on the first target fraction is further defined as producing at least a first amplified nucleic acid comprising the first differentiation domain and a segment of the first nucleic acid target, if the first nucleic acid target is present in the first sample, and at least a second amplified nucleic acid comprising the second differentiation domain and a

segment of the first nucleic acid target, if the first nucleic acid target is present in the second sample.

7. The method of claim 6, wherein the differentiation domain of the first tag and the second tag is appended between the first nucleic acid target sequence and the amplification domain.

8. The method of claim 6, wherein said nucleic acid target is one target of a plurality of nucleic acid targets within the samples.

9. The method of claim 6, wherein said first and second samples are two samples of a plurality of samples.

10. The method of claim 9, wherein the first and second tag are two tags of a plurality of tags.

11. The method of claim 6, wherein the amplification domain of the first nucleic acid tag and the second nucleic acid tag comprises a primer binding domain.

12. The method of claim 6, wherein the amplification domain of the first nucleic acid tag and the second nucleic acid tag comprises a transcription domain.

13. The method of claim 6, wherein the amplification domains of the first and second nucleic acid tags are functionally equivalent.

14. The method of claim 6, wherein the amplification domains of the first and second nucleic acid tags are identical.

15. The method of claim 6, wherein the differentiation domain of the first nucleic acid tag and the second nucleic acid tag comprise at least a size differentiation domain, an affinity domain, or a unique sequence domain.

16. The method of claim 6, wherein said first target fraction is one of a plurality of target fractions.

17. The method of claim 6, wherein the first target fraction is isolated by binding a ligand to at least a segment of the first nucleic acid target.

18. The method of claim 17, wherein the ligand is a nucleic acid, protein, or other molecule with an affinity for certain nucleic acids.

19. The method of claim 18, wherein the ligand is a nucleic acid complementary to at least a segment of the first nucleic acid target.

20. The method of claim 19, wherein the first complementary nucleic acid is used to separate the first target nucleic acid from at least one other nucleic acid or molecule.

21. The method of claim 20, wherein the target fraction is subsequently removed from the first complementary nucleic acid.

22. The method of claim 19, wherein the first complementary nucleic acid is one of a plurality of complementary nucleic acids, and each complementary nucleic acid is complementary to one of a plurality of nucleic acid targets.

23. The method of claim 19, wherein the first complementary nucleic acid is bound to a solid support.

24. The method of claim 23, wherein the first complementary nucleic acid is one of a plurality of complementary acids bound to an array, and each of the complementary nucleic acids is complementary to one of a plurality of nucleic acid targets.

25. The method of claim 23, wherein the solid support is one of a plurality of solid supports.

26. The method of claim 23, wherein the solid support is an array, a microtiter well, a chip, a bead or a combination thereof.

27. The method of claim 6, wherein differentiating comprises binding the first amplified  
5 nucleic acid to a ligand specific to at least a segment of the first differentiation domain or binding the second amplified nucleic acid to at least a segment of the second differentiation domain.

28. The method of claim 6, wherein said differentiation domain of the first nucleic acid tag  
10 comprises a first affinity domain and the second nucleic acid tag comprises a second affinity domain that is distinct from the first affinity domain.

29. The method of claim 28, wherein differentiating comprises binding at least a segment of  
the first affinity domain to a first affinity domain specific ligand and/or binding at least a  
15 segment of the second affinity domain to a second affinity domain specific ligand.

30. The method of claim 27, wherein the first and second affinity domain specific ligands are two of a plurality of ligands.

31. The method of claim 29, wherein at least one of the first or the second affinity domain  
20 specific ligands is bound to at least one solid support.

32. The method of claim 31, wherein the solid support is one of a plurality of solid supports.

33. The method of claim 31, wherein the solid support is an array, a microtiter well, a glass  
25 surface, a chip, a bead or a combination thereof.

34. The method of claim 29, wherein at least one of the first or the second affinity domain  
specific ligands is labeled.

35. The method of claim 29, wherein the binding of the first affinity domain specific ligand to the first affinity domain results in a detectable signal and/or the binding of the second affinity domain specific ligand to the second affinity domain results in a detectable signal.

5 36. The method of claim 35, wherein the binding of the first affinity domain specific ligand to the first affinity domain results in a first detectable signal and the binding of the second affinity domain specific ligand to the second affinity domain results in a second detectable signal.

10 37. The method of claim 36, wherein the first detectable signal is distinguishable from the second detectable signal.

38. The method of claim 6, wherein the differentiation domain of the first nucleic acid tag comprises a first sequence domain and the differentiation domain of the second nucleic acid tag  
15 comprises a second sequence domain that is distinguishable from the first sequence domain.

39. The method of claim 6, wherein differentiating comprises sequencing the first amplified nucleic acid and the second amplified nucleic acid.

20 40. The method of claim 39, wherein the amplified nucleic acid is cloned prior to sequencing.

41. The method of claim 6, wherein the differentiation domain is a unique size domain.

25 42. The method of claim 41, wherein differentiation comprises distinguishing amplified products by size.

43. The method of claim 6, wherein the first nucleic acid tag or the second nucleic acid tag further comprises at least one additional domain.

44. The method of claim 43, wherein said additional domain is a restriction enzyme domain, a secondary amplification domain, a sequencing primer binding site, a labeling domain or a combination thereof.
- 5 45. The method of claim 43, wherein said additional domain comprises one or more restriction enzyme domains.
46. The method of claim 43, wherein said additional domain comprises a labeling domain.
- 10 47. The method of claim 46, wherein the labeling domain is a transcription promoter.
48. The method of claim 46, wherein the labeling domain is a primer binding site.
49. A method of comparing one or more nucleic acid targets within two or more samples,  
15 comprising:
- a) obtaining at least a first sample and a second sample, each potentially having at least a first nucleic acid target;
  - 20 b) preparing at least a first tagged nucleic acid sample by appending at least a first nucleic acid tag comprising a first amplification domain and a first differentiation domain to the first nucleic acid target of the first sample, if the first nucleic acid target is present in the first sample;
  - 25 c) preparing at least a second tagged nucleic acid sample by appending at least a second nucleic acid tag comprising a second amplification domain and a second differentiation domain to the first nucleic acid target of the second sample, if the first nucleic acid target is present in the second sample;
  - 30 d) mixing the first tagged nucleic acid sample and the second tagged nucleic acid sample to create a sample mixture;

- e) isolating at least a first target fraction of the sample mixture;
- f) performing at least a first amplification reaction on the first target fraction, wherein the amplification reaction produces at least a first amplified nucleic acid comprising the first differentiation domain and a segment of the first nucleic acid target, if the first nucleic acid target is present in the first sample, and at least a second amplified nucleic acid comprising the second differentiation domain and a segment of the first nucleic acid target, if the first nucleic acid target is present in the second sample;
- g) differentiating the first amplified nucleic acid in the first target fraction, if any, from the second amplified nucleic acid in the first target fraction, if any; and
- h) comparing the first nucleic acid target of said first sample to the nucleic acid target of said second sample.

50. The method of claim 49, wherein said nucleic acid target is one target of a plurality of nucleic acid targets within the samples.

51. The method of claim 49, wherein said first and second samples are two samples of a plurality of samples.

52. The method of claim 49, wherein the amplification domains of the first and second nucleic acid tags are identical.

53. The method of claim 49, wherein the differentiation domain of the first nucleic acid tag and the second nucleic acid tag comprise at least a size differentiation domain, an affinity domain, or a unique sequence domain.

54. The method of claim 49, wherein the first target fraction is isolated by binding a ligand to at least a segment of the first nucleic acid target.

55. The method of claim 54, wherein the ligand is a first nucleic acid complementary to at least a segment of the first nucleic acid target.

56. The method of claim 54, wherein the first complementary nucleic acid is one of a plurality of complementary acids bound to an array, and each of the complementary nucleic acids is complementary to one of a plurality of nucleic acid targets.

57. The method of claim 49, wherein differentiating comprises binding the first amplified nucleic acid to a ligand specific to at least a segment of the first differentiation domain or binding the second amplified nucleic acid to at least a segment of the second differentiation domain.

58. The method of claim 49, wherein said differentiation domain of the first nucleic acid tag comprises a first affinity domain and the second nucleic acid tag comprises a second affinity domain that is distinct from the first affinity domain.

59. The method of claim 58, wherein differentiating comprises binding at least a segment of the first affinity domain to a first affinity domain specific ligand and/or binding at least a segment of the second affinity domain to a second affinity domain specific ligand.

60. The method of claim 59, wherein at least one of the first or the second affinity domain specific ligands is bound to at least one solid support.

61. The method of claim 59, wherein the binding of the first affinity domain specific ligand to the first affinity domain results in a first detectable signal and the binding of the second affinity domain specific ligand to the second affinity domain results in a second detectable signal.

62. The method of claim 61, wherein the first detectable signal is distinguishable from the second detectable signal.

63. A method of comparing one or more nucleic acid targets within two or more samples comprising

- 5 a) obtaining at least a first sample and a second sample, each potentially having at least a first nucleic acid target;
- 10 b) preparing at least a first tagged nucleic acid sample by appending at least a first nucleic acid tag comprising a first amplification domain and a first differentiation domain to the first nucleic acid target of the first sample, if the first nucleic acid target is present in the first sample, wherein the first differentiation domain comprises a first affinity domain;
- 15 c) preparing at least a second tagged nucleic acid sample by appending at least a second nucleic acid tag comprising a second amplification domain and a second differentiation domain to the first nucleic acid target of the second sample, if the first nucleic acid target is present in the second sample, wherein the first differentiation domain comprises a second affinity domain that is distinct from the first differentiation domain;
- 20 d) mixing the first tagged nucleic acid sample and the second tagged nucleic acid sample to create a sample mixture;
- e) isolating at least a first target fraction of the sample mixture;
- 25 f) performing at least a first amplification reaction on the first target fraction, wherein the amplification reaction produces at least a first amplified nucleic acid comprising the first affinity domain and a segment of the first nucleic acid target, if the first nucleic acid target is present in the first sample, and at least a second amplified nucleic acid comprising the second affinity domain and a segment of  
30 the first nucleic acid target, if the first nucleic acid target is present in the second sample;

- g) differentiating the first amplified nucleic acid in the first target fraction, if any, from the second amplified nucleic acid in the first target fraction, if any, by binding the first affinity domain to a first ligand and the second affinity domain to a second ligand; and

5

- h) comparing the first nucleic acid target of said first sample to the nucleic acid target of said second sample.